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(54) Abstract Title
Removing dung from animal hides

(57) A composition suitable for removing dung from animal hides or skins, including those of live animals, contains at least one enzyme that acts on cellulose, hemicellulose or lignin. In particular, the composition may contain a cellulase and a xylanase and may additionally comprise a laccase. It may also comprise a bactericide, especially when live animals are to be treated, and a thixotropic agent such as pullulan or a styrene-maleic acid or acrylic polymer.The composition may take the form of an aqueous system or a concentrate for dilution with water.

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Removal of Dung

FIELD OF THE INVENTION

This invention is concerned with the removal of dung from animal hides and skins. By conventional usage, hide is the skin of large animals, such as cattle, and skin is the term employed in respect of smaller animals, such as sheep.

The present invention applies to live animals, such as cattle, but more importantly it applies to flayed hides and skins.

BACKGROUND OF THE INVENTION

The presence of dung on animal hides and skins poses a major threat to hygiene in the abattoir. During the flaying procedure, the slaughterman must cut through the dung to effect removal of the hide or skin from the animal. Even if good hygiene practices are maintained in the abattoir, sterile knives are likely to become contaminated with faecal organisms, with consequent infection of the carcass. The outcome is a risk of passing on the organisms to the consumers of the meat.

Whilst there is a general danger of gastro-intestinal problems from *Escherichia coli* infection, there is mortal danger from *E. coli* O157. The appearance of this bacterium in contaminated meat can cause an infectious epidemic, which may be fatal to the weaker members of society, the young and the old. For this reason, a treatment to remove dung from dirty animals in or prior to arrival in the abattoir lairage must be an important contribution to food hygiene.

The cleaning particularly of cattle hides before slaughter is also of interest to the leather industry. Hides are purchased on a weight basis and it is part of the dealing mechanism that an estimate of the weight of dung is made, so that the price can be adjusted accordingly. However, it is undoubtedly the case that tanners are buying some dung at hide prices. Eliminating dung from hides will certainly impact positively on the economics of the leather producing industry.

The presence of dung on hides is perceived to be a problem for tanners during the spring and the summer, because it is at this time that cattle going for slaughter have been over-wintered indoors, fed on high energy diet. It is not known how great a hygiene problem remains, even when the cleaner summer cattle are processed in the abattoir.

Microscopical examination of dung on hide reveals that adhesion is between the dung and the hair alone; there is no sticking of the dung to the epidermis, the outer layer of skin. This accounts for the difficulty in removing dry dung; the matrix of hair within the dung creates a strong composite material. Indeed, mixing wet cattle dung with hair is still used as a building material in the developing economies, in much the same way as the traditional filling in wattle and daub.

Attempts have been made in the past to clean dung from animals before they went into the abattoir. These have ranged from warm showers to scrubbing with stiff brushes; the former was ineffective and the latter was both ineffective and scratched the skin, devaluing the hide.

Likewise, tanners are faced with the problem of dissolving adhering dung in the earliest step in the wet processing, which is soaking. As might be expected, soaking becomes increasingly difficult with drier dung. Dissolution can be achieved by the action of warm water, but the process may take several hours, helped somewhat by the addition of anionic surfactant. Soaking assists in the form of enzyme formulations, usually either proteases or amylases, have been found to be ineffective in this regard, probably because the typical enzymes for this purpose are primarily used to operate on the hide, rather than on dung.

SUMMARY OF THE INVENTION

According to the present invention, a treatment for removing the dung from hides and skins, whether in processing for leather production or on the live animal, is effected by the action of one or more enzymes that target cellulose, hemicellulose and/or lignin.

Thus, the present invention provides a composition suitable for removing dung from animal hides and skins, characterised in that the composition contains at least one enzyme selected from

the group consisting of enzymes that act on cellulose, hemicellulose and lignin. The present invention also provides a method for dung removal, using such a composition.

PREFERRED EMBODIMENTS

The compositions for use in the present method contain one or more enzymes chosen from enzymes that target cellulose, hemicellulose and lignin. The compositions can contain two or three of the enzymes if desired. Typically the compositions take the form of an aqueous system, and may contain further components. The invention also provides concentrates suited for dilution with water to form a composition of this invention.

The enzymes are preferably applied at pH values close to their activity maxima, pH 4-9, preferably pH 5-7. The duration for the treatment can be determined by trial and error, and is preferably no more than 10 hours, better still less than 8 hours. Some mechanical action may be needed to encourage removal of the dung.

In the case of application to live animals, the enzymes can be formulated with a non-cellulosic thixotropic agent, to produce a viscous solution, and optionally a bactericide, preferably a type allowed for food purposes. The treatment of live animals in this way is a non-invasive procedure, and may be carried out in the abattoir, followed for example with a wash which may also serve to remove fresh dung.

A feature of this invention is the specificity of the one or more enzymes that perform the function of dung removal. Surprisingly, it was found that to remove dung, it is necessary to target cellulose and/or hemicellulose, such as by using the enzymes cellulase and xylanase. Cellulases and hemicellulases act as a complex synergistic mixture, depolymerising and hydrolysing polysaccharides to glucose, xylose and other monosaccharides.

In addition, lignin must usually also be addressed. Lignases and polyphenol oxidases degrade lignin oxidatively, in a non-specific mechanism, by the generation of cation and other radicals in the substrate. Degradation of lignin probably enables access to cellulose and hemicellulose

polymers and their degradation by cellulases and hemicellulases; the latter also disrupts the covalent bonding of hemicellulose to lignin.

Another feature of this invention is the production of optimum mixtures of enzymes for this purpose by the controlled growth of wood rotting or lignolytic fungi: these include *Trichoderma viride*, *Coriolus versicolor*, *Pyricularia oryzae*. Such organisms secrete different enzymes, depending on the growing conditions of substrate, nutrients and atmosphere. Any treatment applied to live animals may involve either a concentrated preparation of the crude incubated organism, containing both organisms and extracellular enzymes, or a mixture of isolated and purified enzymes.

When the treatment is applied to the live animal, there might be a concern for hygiene in presenting a wet animal to slaughter. Bacterial growth is favoured by wet conditions, such that the relatively dry state of the carcass is less conducive to bacterial growth than a wetted or washed carcass. Consequently, in some cases, it may be necessary to incorporate into the treatment some bactericidal activity, to ensure adequate sterility of the hairs. There are three primary requirements of a bactericide in this context: first, it must be compatible with food for human consumption, in case of carry over onto the carcass; second, it must be stable in the presence of the enzyme formulation, so that its activity is maintained under the conditions of storage; third, it must not adversely affect the enzyme activity, either during storage or during the treatment. Examples of possible agents are: bronopol (2-bromo-2-nitro-propan-1,3-diol), biguanide or dithiocarbamate bactericides.

The application of a suitable enzyme or mixture of enzymes to live animals may have to include a mechanism for maintaining contact between the enzyme and the dung for a period long enough for the reaction to take place to an extent sufficient to allow easy rinsing. For example, the enzyme can be formulated to make a viscous preparation which can be painted on the live animal. The thixotropic agent may be of any chemical type that is unaffected by the enzymes or an optional bactericide. In the case of enzyme action, clearly the modern generation of cellulosic polymers would be unsuitable, but other polymers could be used, such as pullulan, styrene- maleic acid or acrylic polymers.

An enzyme preparation may be applied both to flayed hides and skins, or live animals, at any suitable point. Flayed hides are commonly treated with salt (NaCl), in order to preserve the hide. As shown herein, the enzymatic activity of cellulase and xylanase is increased in the presence of NaCl, at least in the range 0 - 3M. Therefore, the enzyme treatment may be carried out in the first wash of the hides in tanneries, such that dung is removed from the hide at the first stage in leather processing.

EXAMPLES OF THE INVENTION

This invention is further illustrated by the following examples.

Example 1. The effects of different enzymes on dung removal.

5 cm^2 pieces of dung clad hide were treated with different enzymes, using pH buffers at pH 5 or 7, depending on the pH optima. 10 ml volumes of each enzyme were used, containing approximately equal activity based on rate of production of reducing sugars, 50-100 units. (In this context, one unit is defined as the quantity of enzyme that will liberate 1 mole of reducing sugar, measured as xylose equivalents, from xylan per minute at pH 4.5 and 30°C.] Samples were agitated by rotatory shaker at ambient temperature overnight, then ease of dung removal was determined. The result was estimated by passing a spatula over the hair surface and giving a score using the following scale.

***	easy removal
**	removed with moderate difficulty
*	difficult and incomplete removal
0	no appreciable removal

enzyme	pH	score
Cellulase	5.0	***
Xylanase (hemi-cellulase)	5.0	***
Laminarinase (β-1,3-glucanase)*[a]	5.0	**
β-galactosidase	5.0	*

Control, no enzyme	5.0	0
α-amylase	7.0	*
mannosidase	7.0	*
β-glucosidase	7.0	*
Protease	7.0	*
Control, no enzyme	7.0	0

*a at 50x concentration.

Where the enzymes were effective, the dung was removed leaving the hairs intact and apparently very clean and silky.

Example 2. The effect of enzyme concentration on dung removal.

The experimental conditions were the same as described in Example 1.

enzyme conc. (units)	cellulase	xylanase
50	***	***
25	***	**
10	*	*
1	*	*
0	0	0

Example 3. The rate of dung removal.

The experimental conditions were the same as described in Example 1.

Reaction time (hours)	6	12	14	16	18	20	24
Cellulase: 50 units	*	*	**	**	***	***	***
Cellulase: 25 units	*	*	**	**	***	***	***
Xylanase: 50 units		*	**	**	**	***	***
Xylanase: 25 units		*	*	**	**	**	***

These results demonstrate that cellulase reacts faster than xylanase at ambient temperature. Also, at 25-50 units concentration, the threshold reactivity of cellulase is less concentration dependent than xylanase.

The preferred enzyme mixture, for the optimal removal of dung within 8 hours at 25°C - 35°C, is 50 units of cellulase with 10 units of xylanase.

Example 4. The influence of ligninase on dung removal.

The experimental conditions were the same as described in Example 1. In these trials, a ligninase, polyphenol oxidase or laccase, was used; in this context, 1 unit of enzyme activity is defined as a change in absorbance at 530 nm of 0.001 optical density units per minute at pH 6.5 and 30°C, using syringaldazine as substrate. Combining laccase with cellulase and xylanase enhanced the reaction.

Enzyme and concentration	Score
0.16 units laccase	**
0.08 units laccase 50 units cellulase 50 units xylanase	****[#b]
0.08 units laccase 25 units cellulase 25 units xylanase	***

[#b] this score goes outside the scale defined in Example 1, because the dung was removed without the need for any appreciable mechanical action.

Example 5. The effect of NaCl on enzyme activity

The effect of salt concentration on enzyme activity was assessed. The definition of enzyme activity units is that given in Example 1.

Salt concentration	Cellulase activity (U)	Xylanase activity (U)
enzyme in buffer	5.3	12
enzyme in tap water	5.6	18
0.2M	5.6	15
0.4M	5.1	14
0.6M	5.5	15
1.0M	6.0	16
1.5M	6.2	17
2.0M	6.2	23
2.5M	6.4	24
3.0M	6.3	25

Claims:

1 The use of at least one enzyme selected from the group consisting of enzymes that act on cellulose, hemicellulose and lignin, in the preparation of a composition suitable for removing dung from animal hides and skins.

2 The use according to claim 1, wherein the composition contains a cellulase and a xylanase.

3 The use according to claim 2, wherein the composition contains cellulase and xylanase in the ratio of 5:1.

4 The use according to claim 1, wherein the composition contains a laccase.

5 A method for removing the dung from hides and skins, characterised in that the [illegible] treated with a composition containing at least one enzyme selected from the group consisting of enzymes that act on cellulose, hemicellulose and lignin.

6 A method according to claim 5, wherein the composition contains a cellulase and a xylanase.

7 A method according to claim 6, wherein the composition contains cellulase and xylanase in the ratio of 5:1.

8 A method according to claim 6, wherein the composition contains a laccase.

9 A method according to any of claims 5 to 8, wherein the duration of the treatment is less than 8 hours.

10 A method according to any of claims 5 to 9, wherein the treatment is carried out in the first wash of the hides.

The Patent Office

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Claims searched: 5-10

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.P): C6C (C2)

Int Cl (Ed.6): C14C 1/00, 1/06

Other: Online: WPI

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
A	GB 2233665 A	(RÖHM GmbH) see p.2 l.11 - p.3 l.12	5
A	US 4202664	(GIMELFARB ET AL.) see col.1 l.15 - col.2 l.27	5

X	Document indicating lack of novelty or inventive step	A	Document indicating technological background and/or state of the art.
Y	Document indicating lack of inventive step if combined with one or more other documents of same category.	P	Document published on or after the declared priority date but before the filing date of this invention.
&	Member of the same patent family	E	Patent document published on or after, but with priority date earlier than, the filing date of this application.